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Journal

Journal of Analytical Toxicology, 40(7)

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Publication Date

2016-09-01

DOI

10.1093/jat/bkw041

Peer reviewed

Journal of Analytical Toxicology, 2016;40:511–518
doi: 10.1093/jat/bkw041
Advance Access Publication Date: 21 July 2016
Article

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Quantification of Polybrominated and Polychlorinated Biphenyls in Human Matrices by Isotope-Dilution Gas Chromatography–Tandem Mass Spectrometry

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Abstract

We have developed a highly sensitive and selective analytical method capable of quantifying a total of 15 polybrominated and polychlorinated biphenyls (11 PBBs and 4 PCBs) in human serum. Samples were subjected to liquid–liquid extraction followed by solid-phase extraction prior to measurement using gas chromatography–tandem mass spectrometry in multiple reaction monitoring mode. Quantification was performed using isotope-dilution calibration covering a concentration range of 0.005–12.5 ng/mL. Limits of detection for all target compounds were in the low range (0.7–6.5 pg/mL). The method was validated using in-house pooled human serum fortified at two concentrations (0.5 ng/mL and 1.0 ng/mL), whole semen fortified at one concentration (0.25 ng/mL), and NIST Standard Reference Material (SRM) 1958, which includes five of the target compounds. Method accuracies for all target compounds ranged from 84 to 119% with relative standard deviations (RSDs) of <19%. The measured values for the five target compounds present in the SRM agreed with the certified reference values (89–119% accuracy with RSDs <9%). As this method was developed to support ongoing epidemiologic investigations, we evaluated its suitability by analyzing subsets of serum and whole semen samples from the Michigan PBB Registry cohort. PBB-153, PCB-118, PCB-138, PCB-153 and PCB-180 were detected in all serum samples analyzed, with PBB-77 and PBB-101 detected less frequently in serum. PBB-153, PCB-118, PCB-138, PCB-153 and PCB-180 were detected in at least one whole semen sample.

Introduction

Polybrominated biphenyls (PBBs) and polychlorinated biphenyls (PCBs) are persistent organic pollutants associated with adverse human health effects. PBBs and PCBs share a basic structure consisting of two phenyl rings with halogen substitution; congeners, which differ in the number (1–10) and position of the respective halogen substitution (1), have either planar or non-planar configuration dependent upon degree of substitution in the ortho positions, which affects the degree of rotation around the central carbon bond. This geometry, in addition to the overall degree and type of halogen substitution, gives rise to differences in molecular structure. Such differences result in congener-specific interactions with physiologic targets and ultimately affect biological persistence and toxicity. PBBs and PCBs interact with a variety of receptors and disrupt normal hormone function, induce liver enzymes and suppress the immune system. Exposure to these compounds has been linked to adverse reproductive outcomes and cancer (2, 3).

PCBs have been more widely studied than their brominated analogs, in part, because PCBs were produced for decades longer and

in quantities order of magnitudes greater than PBBs. In 2013, the International Agency for Research on Cancer (IARC) re-evaluated both classes of polyhalogenated biphenyls resulting in reclassifications of PCBs as Group 1 "carcinogens" and PBBs as Group 2 A "probably carcinogenic to humans" (4). Both classes continue to persist in the environment and in human populations, despite manufacture and distribution having been mostly phased out for decades. Analyses of representative samples from the 2003–2004 National Health and Nutrition Examination Survey (NHANES) found detectable concentrations of the PCBs included in our study (PCBs 118, 138, 153 and 180) in all samples (5). PBB-153, the predominant congener in hexabrominated biphenyl commercial mixtures, was detected in 83% of the samples from the same survey population (5, 6).

PBBs are brominated flame retardants (BFRs), with similarities in structure to the polybrominated diphenyl ethers and subsequent generations of BFRs. Given concerns of toxic effects of exposure to BFRs (7, 8), there is interest in exposure-related health effects and mechanisms of toxicity of PBBs. Much of our understanding of the human health effects and toxicity of PBBs evolved from an agricultural contamination incident in the state of Michigan. In this incident, a technical mixture of PBBs, FireMaster FF-1, was unintentionally mixed with animal feed in place of a nutritional supplement, which resulted in widespread contamination of Michigan livestock. Ultimately, the Michigan residents were exposed to these compounds through consumption of animal products. In addition to toxicological studies, many of which were conducted with technical mixtures that limit interpretability, the Michigan Department of Health and Human Services (MDHHS) established a research registry to monitor long-term health effects (9).

Research continues to date with this population; however, this has been limited by available exposure data, which consists of biological measurements made in the late 1970s through the early 1990s using gas chromatography with electron-capture detection (GC-ECD). Over time, this method utilized different materials for quantification, from technical flame retardant mixtures to standard materials in the early 1990s but consistently determined only the predominant congener, PBB-153, for which the limit of detection (LOD) was reported as 10 ng (10, 11). Not only were a large proportion of the reported data below the LOD but this also precluded evaluation of individual congener associations, which may contribute differently to toxicity and to related health outcomes. Even with such limited exposure characterization, research with this population has demonstrated intergenerational transfer of PBBs (12) and exposure-related health effects including poorer neonatal health (13), adverse reproductive outcomes (14), menstrual irregularities (15), accelerated pubertal development among females (16), increased incidence of urogenital problems (17), slower growth among males (18) and risk of digestive cancer and lymphoma (19) as well as a non-statistically significant increased incidence of breast cancer (19, 20).

Improvements in analytical approaches and technology now allow for better assessment of exposure to these compounds, thus we developed a highly sensitive and selective analytical method for the quantification of select PBBs and PCBs in human serum using gas chromatography with electron ionization-tandem mass spectrometry (GC–EI–MS-MS).

Experimental

Chemicals and consumables

Analytical grade dichloromethane, hexane and isooctane were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Water was generated using a Milli-Q Ultrapure water purification system (Millipore, Billerica, MA). Formic acid and sulfuric acid were obtained from Fisher Scientific (Lawn, NJ). Bondesil silica sorbent was purchased from Agilent (Santa Cruz, CA), and Isolute® 200 mg silica cartridges were purchased from Biotage (Charlotte, NC). Anhydrous sodium sulfate was obtained from Sigma-Aldrich. Pooled human serum was obtained from the Red Cross (Interstate Blood Bank, LLC, Memphis, TN). The standard reference material (SRM 1958, organic contaminants in fortified human serum) was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD). Helium and nitrogen gas were of 99.999% ultra-high purity and obtained from nexAir, Inc. (Suwanee, GA).

The purity of all PBB and PCB native standards was ≥95%. PBB congeners 15, 18, 52, 101 and 180 were purchased as individual congeners at 50 µg/mL in nonane/toluene from Wellington (Guelph, Ontario, Canada). PBB congeners 80 and 103 were purchased as individual congeners at 100 µg/mL in hexane from ULTRA Scientific (N. Kingstown, RI). PBB congeners 77, 126, 153 and 157, as well as PCB congeners 118, 153, 138 and 180 were purchased from Cambridge Isotope Laboratories (Andover, MA) as individual congeners at 100 µg/mL in isooctane. An individual PBB-153 ^{13}C-ring labeled standard at 40 µg/mL in nonane (99% purity) and a mixed PCB congener ^{13}C-ring labeled standard at 5 µg/mL in nonane (98% purity) were also purchased from Cambridge Isotope Laboratories.

Preparation of standard solution and quality control materials

Native PBB-PCB mixed calibration standards (purity-adjusted) were prepared by serial dilution of 0.5 ng/µL stock solution in acetonitrile: dichloromethane (4:1). All calibrants were solvent-based standards with each concentration expressed in serum equivalents. For example, the highest calibrant concentration was equivalent into that in the final extract from a serum sample whose concentration was 12.5 ng/mL. By expressing the concentrations in serum equivalents, our overall quantification scheme was simplified. Calibration standards serum equivalent concentrations ranged 0.005–12.5 ng/mL. A labeled standard spiking solution was prepared at a concentration of 0.2 µg/mL in acetonitrile:dichloromethane (4:1).

Two quality control (QC) spiking solutions containing the native mixture were prepared with standard spiking solutions by serial dilution of the initial stock solution. When spiked into serum matrix, the nominal concentrations of the matrix-based QC samples were 0.5 and 1.0 ng/mL. All standard stock solution and spiking solutions were dispensed into amber vials and stored at 4°C until used.

Extraction

Each sample (1 mL serum) was spiked with 50 µL labeled standard solution resulting in a 10 ng/mL internal standard concentration. Each sample was then vortex mixed briefly before adding 2 mL of a formic acid:water solution (50:50). Each sample was vortex mixed briefly again, and then 5 mL of hexane was added to each sample. Samples were sonicated for 1 minute and then vortex mixed at 2000 rpm for 10 minutes using a multivortexer (Benchmark BenchMixer, Edison, NJ). Samples were centrifuged, and the organic layer was removed by Pasteur pipette to a clean test tube. An additional 5 mL of hexane was added to each sample, with the sonication, vortex mixing and centrifugation repeated as above, and with the resulting organic layer being pulled off and added to the first.

Following this liquid–liquid extraction, a cleanup process using solid-phase extraction was performed using in-house prepared acidified

silica added to silica cartridges. Acidified silica (silica/sulfuric acid 2:1 by weight) was prepared by the addition of concentrated sulfuric acid to Bondesil silica and heating at 100°C overnight. Isolute cartridges (10 mL capacity, 200 mg silica) were then packed with 1.8 g of this material and topped off with 0.5 g of anhydrous sodium sulfate. These prepared acidified silica/silica cartridges were then conditioned with 5 mL hexane immediately prior to sample cleanup. Samples were loaded to the cartridges, and sample breakthrough was collected. Sample tubes were rinsed with 1 mL of hexane, briefly vortex mixed and shaken, and also loaded to the cartridge. After samples finished loading, the breakthrough collection tubes were inserted into the TurboVap® (Zymark, Framingham, MA) set at 30°C and 15 psi to begin evaporation. Cartridges were then eluted with 10 mL of a 1:19 dichloromethane:hexane solution. Eluate was collected and combined with the breakthrough already evaporating and brought to total dryness in the TurboVap®. Samples were reconstituted with 50 µL of isooctane for instrumental analysis.

GC–MS-MS analysis

Analysis was performed by GC–MS-MS using an Agilent 7890 A gas chromatograph coupled to an Agilent 7000B tandem mass spectrometer (Agilent Technologies, Santa Clara, CA). The system was programmed and controlled using MassHunter Workstation Software version B.05.00. Calibration and tune of the instrument was performed in the EI with High Sensitivity Autotune mode, and instrumental performance was always checked prior to analysis.

The GC system was fitted with a polyimide-coated fused silica phase analytical column (15 m × 0.250 ID × 0.10 µm film thickness, ZB-5HT Inferno; Phenomenex, Torrance, CA) for an optimum separation. A 2 µL injection was used with an injection port temperature set to 325°C under pulsed splitless mode. The helium carrier gas flow rate was 2.25 mL/minute through the end of the run, with a quench nitrogen gas flow rate of 1.5 mL/minute. The oven temperature program was as follows: 90°C (0.1 minute), ramped to 340°C (20°C/minute) and held for 5 minutes. The total run time was 17.6 minutes. Source and quadrupole temperatures were set to 230 and 150°C, respectively.

To create the MS/MS quantification method, individual injections of each target compound in full-scan mode were done in order to obtain their retention times and to select the optimal precursor ions, generally selecting the most intense ion with the highest *m/z*. Product ion scan was performed using different collision energies set to determine the most selective products ions. Ions were selected based on their intensity, peak shape and signal-to-noise (S/N) ratio. Two transitions were then selected for each native analyte for quantification and confirmation. Only one transition was selected for each labeled analyte, ^{13}C-PBB-153, ^{13}C-PCB-118, ^{13}C-PCB-138, ^{13}C-PCB-153, and ^{13}C-PBB-180. All transitions were monitored in a multi-segment analysis using multiple reaction monitoring (MRM) mode, with wide resolution for MS1 and unit resolution for MS2. These MRM transitions and associated parameters are summarized in Table I.

Data processing

Data were processed using MassHunter Workstation Software—Quantitative Analysis version B.05.00. Generally, each compound was characterized by its retention time, S/N ratio, relative retention time value, peak algorithm, quantitation transition and confirmation transition.

Validation study

This method was validated based on an in-house protocol using pooled human serum. Validation of the method involved multiple criteria, including optimum extraction recovery, acceptable precision and accuracy and linearity as described here.

Extraction recovery

The extraction recovery of the method was determined at one spiked concentration (0.5 ng/mL) using pooled human serum. We first spiked each of five serum samples (1 mL) with mixtures of native and labeled compounds and extracted according to the method. We

Table I. MRM transitions and related parameters by target compound

Target compounds (Ballschmiter–Zell nomenclature)	Retention time (minutes)	MRM transition 1	Collision energy 1 (eV)	MRM transition 2	Collision energy 2 (eV)
PBB-15	4.8	311.8 → 152.1	30	309.8 → 152.1	30
PBB-18	4.8	310.8 → 230	20	310.8 → 232	20
PBB-52	6.3	309.8 → 150.1	35	388.8 → 309.8	35
PBB-77	7.6	309.8 → 150.1	45	469.8 → 309.8	30
PBB-80	7	309.8 → 150.3	45	469.2 → 309.9	35
PBB-101	7.6	468.8 → 389.8	25	387.7 → 227.9	55
PBB-103	7	469.2 → 230	60	547.9 → 387.8	40
PBB-126	8.55	387.7 → 228	35	547.8 → 387.9	30
PBB-153	8.55	467.8 → 307.9	40	627.9 → 467.8	45
PBB-153 (IS)	8.55	479.8 → 319.9	40		
PBB-157	9.8	307.8 → 148.1	30	627.9 → 467.6	40
PBB-180	10.25	385.8 → 147.1	60	627.0 → 545.6	30
PCB-118	5.9	323.7 → 254	30	325.7 → 256	25
PCB-138	6.55	359.7 → 289.9	30	359.7 → 279.9	30
PCB-138 (IS)	6.55	371.7 → 301.9	30		
PCB-153	6.3	359.7 → 289.9	40	289.7 → 218	40
PCB-153 (IS)	6.3	371.7 → 301.9	40		
PCB-180	7	393.7 → 323.9	30	323.7 → 254	45
PCB-180 (IS)	7	405.7 → 335.9	30		

also concurrently extracted an additional five pooled serum samples spiked only with internal standard, deviating from the method only in that, prior to evaporation, each of these five extracts were spiked with the same mixture of native standards mentioned earlier. Thus, these samples represented 100% recovery. All samples were reconstituted and analyzed immediately after evaporation. Recovery was calculated by comparing the responses of the pooled serum samples spiked before extraction to the responses of the pooled serum samples spiked after the extraction.

Precision

We determined the method precision by calculating the RSD of repeat measurements of pooled serum samples spiked with QC materials at two different concentrations (0.5 and 1.0 ng/mL). Inter-day precision was determined using QC materials prepared and analyzed during a discontinuous sample analysis period over 2 months ($n = 5$ for each concentration).

Accuracy

For all compounds, we determined method accuracy by calculating the difference in the mean of repeat measurements of pooled human serum ($n = 5$) spiked with mixtures of native compounds at two concentrations (0.5 and 1.0 ng/mL) from the expected concentration. For PBB-153 and the four PCB congeners, we also reported percentage of agreement between the mean quantified values of the NIST certified reference serum ($n = 4$) generated from our method and their specified mean values for each lot of the reference materials.

Limit of detection

LOD is defined as the concentration at which the S/N ratio of the observed signal was ≥3. These values were extrapolated from the S/N ratio of the lowest standard concentrations of each compound with appropriate chromatographic characteristics (e.g., peak shape, retention time, relative retention time). The extrapolated LODs were confirmed visually by the injection of a standard at or near the LOD.

Results

Chromatographic separation

Regarding selectivity of the method, pooled serum samples (served as method "blank" samples) showed no isobaric or chromatographic interferences with target compounds. Figure 1 shows the extracted ion chromatograms representing target native compounds from analysis of a blank serum sample, a typical 1.0 ng/mL fortified pooled serum sample and a representative unknown sample.

Linearity and LODs

Our calibration curve was linear at a range from 0.005 to 12.5 ng/mL with correlation coefficients (r) between 0.994 and 0.998. The errors about the slope for all compounds were <1.5% with the exception of PBB-15 (3.1%). Estimated detection limits were in the low pg/mL, ranging from 0.7 to 6.5 pg/mL across target compounds. Estimated detection limits are presented in Table II.

Extraction recovery

The results of our extraction recovery are presented in Table II. All target compounds were shown to have good recovery using our extraction method, with calculated recoveries ranging from 83.2 to 99.2%. The majority of the target compound recoveries were

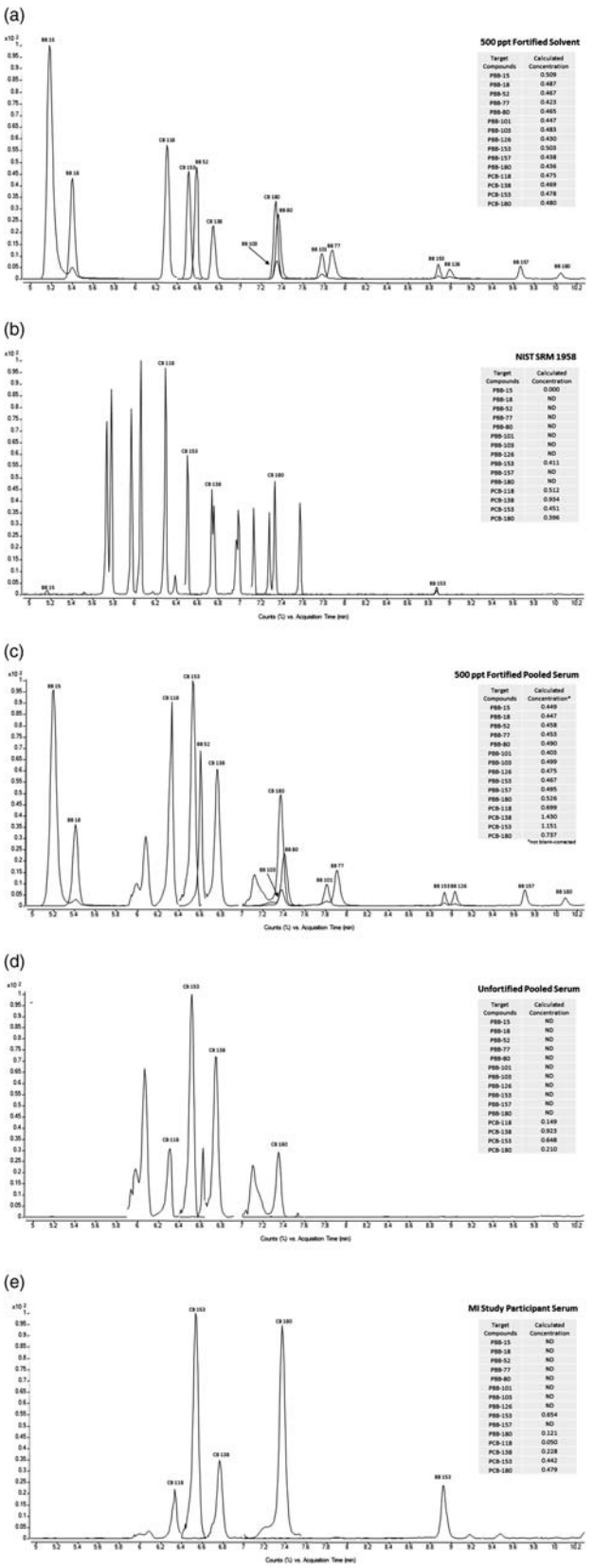

Figure 1. Representative extracted ion chromatograms of target native compounds. (a) 500 ppt fortified solvent. (b) NIST SRM 1958. (c) 500 ppt fortified pooled serum. (d) Unfortified pooled serum. (e) Sample from an Michigan study participant.

Table II. Fortified serum validation: in-house material validation including extraction recovery, mean response, accuracy and precision (RSD) of target halogenated biphenyls with additional NIST reference material validation with accuracy and precision (RSD) for applicable target compounds

Target compounds	Spiked concentrations (ng/mL)	Extraction recovery (%)	Mean concentration (ng/mL)	Accuracy (%)	RSD (%)	LOD (pg/mL)
PBB-15	0.5	85.0	0.489	97.9	12.7	2.6
	1		1.003	100.3	16.5	
PBB-18	0.5	83.2	0.474	94.8	11.1	0.8
	1		1.003	100.3	16.5	
PBB-52	0.5	88.4	0.508	101.6	4.2	2.2
	1		0.982	98.2	3.7	
PBB-77	0.5	90.4	0.519	103.9	13.4	4.5
	1		1.065	106.5	10.9	
PBB-80	0.5	94.9	0.527	105.4	2.2	1.7
	1		1.083	108.3	4.7	
PBB-101	0.5	92.1	0.429	85.7	16.3	3.9
	1		0.848	84.8	15.8	
PBB-103	0.5	96.1	0.453	90.6	18.4	2.3
	1		0.932	93.2	15.7	
PBB-126	0.5	94.0	0.512	102.4	9.0	6.5
	1		1.060	106.0	7.8	
PBB-153	0.5	97.1	0.457	91.4	7.4	2
	1		0.873	87.3	4.8	
	Certified NIST SRM 1958 (0.421 ± 0.013)		0.397	94.3	8.0	
PBB-157	0.5	91.3	0.532	106.5	8.9	3.7
	1		1.127	112.7	5.1	
PBB-180	0.5	89.5	0.571	114.1	3.5	5.6
	1		1.181	118.1	7.7	
PCB-118	0.5	93.1	0.468	93.6	12.8	1.4
	1		0.846	84.6	3.1	
	Certified NIST SRM 1958 (0.412 ± 0.035)		0.489	118.8	8.5	
PCB-138	0.5	99.2	0.457	91.5	16.1	1.2
	1		0.858	85.8	5.0	
	Certified NIST SRM 1958 (0.473 ± 0.054)		0.459	97.0	3.5	
PCB-153	0.5	97.2	0.451	90.1	9.2	1.6
	1		0.881	88.1	7.6	
	Certified NIST SRM 1958 (0.457 ± 0.036)		0.441	96.6	2.8	
PCB-180	0.5	97.6	0.448	89.7	12.5	0.7
	1		0.843	84.3	6.2	
	Certified NIST SRM 1958 (0.459 ± 0.049)		0.409	89.1	5.3	

>90%, with lowest recoveries occurring with the di- and tri- substituted bromobiphenyls.

Accuracy and precision

The validation results using two concentrations of fortified pooled human serum are shown in Table II. Analyses of these fortified samples occurred in different batches across 6 months. For all 15 of the target compounds, accuracies ranged from 84.3 to 118.1%. Inter-day precision, expressed as the percent RSD, ranged from 2.2 to 18.4% across target compounds, with a majority of target compounds having precision <15% at both fortification concentrations. Overall, method accuracy and precision for all target compounds meet the US Food and Drug Administration guidance recommendation criteria for method accuracy (80–120%) and method precision (<20%) in bioanalytical method development (21). This is further supported by the results of our analysis of NIST SRM 1958 replicates ($n = 4$) in which mean measured values for the subset of five target compounds present in SRM 1958 agree with the certified reference values with accuracy ranging from 89 to 119% and precision ranging from 2.8 to 8.5%.

Application to unknown samples

To demonstrate the applicability of the developed method to real-world samples, we analyzed a subset of biological samples collected as part of ongoing research with the Michigan PBB Registry cohort. Blood samples were collected by venipuncture and processed using standard protocols to obtain serum, which was then stored at −20°C until analysis. Twenty-five of these serum samples were extracted as described here, along with QC samples including a pooled serum matrix blank and two concentrations of fortified pooled serum. This preliminary data are presented in Table III. PBB-153 and all four PCB congeners (118, 138, 153 and 180) were detected in every sample. PBB-77 and PBB-101 were detected in a small fraction of the samples.

Extension to other matrices

Although this method was developed for use with serum, plasma and whole semen collected as part of the Michigan study have also been analyzed. Plasma is generally considered to be interchangeable with serum in such analyses; our analysis of serum and plasma samples for a subset of individuals with both biological samples available (agreement >97%) supports this. Whole semen, however, is sufficiently

dissimilar in composition to serum that prior to analysis of whole semen from exposed individuals, we conducted an abbreviated validation experiment with this matrix for a subset of target compounds (PCBs 118, 138, 153 and 180 as well as PBB-153). This abbreviated extraction recovery was determined at one spiked concentration (0.25 ng/mL) using pooled whole semen from unexposed individuals. We first spiked each of four semen samples (0.3 mL) with mixtures of native and labeled compounds and extracted according to the method. We also concurrently extracted an additional four pooled whole semen samples spiked only with internal standard, deviating from the method only in that, prior to evaporation, each of these four extracts were spiked with the same mixture of native standards mentioned earlier, so as to represent 100% recovery. All samples were reconstituted and analyzed immediately after evaporation. As in the serum extraction recovery experiment, recovery was calculated by comparing the responses of the pooled serum samples spiked before extraction to the responses of the pooled serum samples spiked after the extraction. Recoveries ranged from 94.2 to 97.2%, comparable to those of serum for these compounds (Table IV). Accuracies ranged from 90.4 to 104.9%, with precisions (RSD) from 7.7 to 12.7%. Given these results, our method was determined to be suitable for use with this matrix, such that we proceeded to analyze a set of five individuals from the Michigan PBB Research Registry with at least 300 µL whole semen available. Of these, one individual had detectable levels of all five target compounds.

Discussion

There is considerable interest in the evaluation of environmental and human health effects associated with exposure to PBBs. Despite this, few modern analytical methods have been specifically developed for analysis of PBBs in human biological samples or fully validated for inclusion of PBBs in an existing method, the latter of which is the case in several studies with PBBs, or just PBB-153, included in an existing method for PBDEs, PCBs or other related compounds.

Table III. Target compounds measured (pg/mL) in Michigan PBB Registry cohort serum ($n = 25$)

Target compounds	Detection frequency	Median concentrations (pg/mL)	Range (pg/mL)
PBB-15	ND		
PBB-18	ND		
PBB-52	ND		
PBB-77	8%	190	67–312
PBB-80	ND		
PBB-101	8%	240	121–358
PBB-103	ND		
PBB-126	ND		
PBB-153	100%	665	28–236,064
PBB-157	ND		
PBB-180	ND		
PCB-118	100%	62	20–305
PCB-138	100%	175	46–797
PCB-153	100%	191	43–970
PCB-180	100%	223	28–848

PBB exposures in human populations have been reported using three approaches in recent years. GC-ECD, while relatively inexpensive, is the same approach used for quantification in the early Michigan studies and although modern capillary columns have improved separation, GC-ECD is still subject to many limitations in terms of identification, including the potential for false positives due to interferences. Additionally, co-elution of congeners with different masses, as well as other compounds of interest, such as PBDEs or PCBs may also be an issue in GC-ECD. However, GC-ECD has been used recently for quantification of three PBB congeners in serum (22). GC–MS offers some improvements over GC-ECD in terms of selectivity with the addition of an additional mass-to-charge ratio as means for identification and confirmation although it is also subject to some degree of interference. GC–MS also allows for the application of isotope-dilution techniques that further increases selectivity. GC–MS has been used for quantification of PBBs in three recent studies (23–25); however, limited method performance or validation data were reported.

More modern instrumental approaches have also been employed for PBB exposure in human subjects. gas chromatograph-high resolution mass spectrometry (GC-HRMS) is considered the most selective and specific approach used to date although it is more costly, less robust and requires more specialized training as compared to GC–MS-MS. Four studies (26–29) have utilized GC-HRMS but only one had significant method development and validation information available (6, 26). Sjödin *et al.* (6) reported an extraction and cleanup method for measurement of PBBs, PBDEs and PCBs in human serum, and included precision and recovery data as well as congener-specific LODs. This method only included a single PBB congener, 153, but made use of a direct isotopically labeled internal standard that allows for optimal analytical precision. With this method, the LOD was reported as 1 pg/mL. The only study to include multiple PBB congeners in a GC-HRMS method was a study of persistent organohalogens in breast milk and placenta in Denmark and Sweden (27), which included 17 congeners but reported no validation data.

The only previous study utilizing GC–MS-MS for the determination of PBB exposure was a recent investigation of emerging and legacy BFRs in paired maternal breast milk and serum (30). In this study, determination of two PBB congeners, PBB-153, PBB-209, and six additional BFR compounds was performed using electron-capture negative chemical ionization mode with peak identification and quantification by selected ion monitoring. However, limited method performance or validation data was provided other than internal standard recoveries, LOD and duplicate sample agreement. Average percent recovery and standard deviation were reported for the PBB-153 surrogate, $_{13}C_{12}$ PBDE-153, as 50 ± 18, and for the PBB-209 surrogate, $_{13}C_{12}$ PBDE-209, as 33 ± 22. LOD for PBB-153 was

Table IV. Validation data for five target compounds in semen

Target compounds	Spiked concentrations (ng/mL)	Extraction recovery (%)	Mean concentrations (ng/mL)	Accuracy (%)	RSD (%)
PBB-153	0.25	97.2	0.247	98.6	7.7
PCB-118	0.25	94.5	0.262	104.9	9.8
PCB-138	0.25	94.9	0.240	95.9	8.4
PCB-153	0.25	94.2	0.235	93.9	8.8
PCB-180	0.25	94.6	0.226	90.4	12.7

extrapolated from the lowest detected lipid-adjusted concentration in samples and reported as 0.01 ng/g. LOD for PBB-209 was derived from the lowest standard concentration that produced S/N ratio ≥3, and was reported as 0.49 ng/g. Paired sample duplicates ($n = 18$) were reported to have a mean percent difference of 13% for PBB-153 (30).

Each of these existing methods fails to address both of the previously identified major limitations in exposure characterization for epidemiologic research on PBB exposure, improvement in LOD and unequivocal determination of multiple congeners. Our method is comparable in performance in terms of LOD for the best existing method (26), and allows for quantification of multiple additional congeners with confidence, given method accuracies for all target compounds that range from 84 to 119% with RSDs <19%. Because individual congeners may contribute differently to toxicity, previous methods are insufficient to characterize human exposure for the evaluation of human health effects. Our approach using isotope-dilution GC–EI–MS-MS improves selectivity and sensitivity through MRM of unique transitions in a novel approach to PBB and PCB congener identification for human biomonitoring.

Conclusion

We successfully developed a GC–MS-MS method that is highly sensitive, highly selective and reproducible for quantification of PBBs and PCBs in human sera as well as plasma and whole semen. This method is suitable for the detection of these persistent compounds over a range of serum concentrations from background to those resulting from specific exposure scenarios, such as those related to the 1973 Michigan PBB incident.

Acknowledgments

The authors would like to thank the members of the Michigan PBB Registry for their interest and participation. We further thank the Emory Michigan PBB Registry team and the team of the Laboratory of Exposure Assessment and Development in Environmental Research (LEADER) within the Rollins School of Public Health and the HERCULES Analytic Core.

Funding

This work was supported by NIEHS research grants 2R01ES012014 and R21ES023927, NIH Grant 5RC1ES01829902 under the American Recovery and Reinvestment Act of 2009, NIEHS training grant T32ES012870 and NIEHS center grant P30ES019776, P30ES01977603S1, P30ES01977603S2. Michigan PBB Registry research has also been supported by the US Centers for Disease Control and Prevention and the US Environmental Protection Agency. This work was also supported, in part, by a grant from the Burroughs Wellcome Fund supporting the Molecules-to-Mankind graduate program at Emory University. Dr Panuwet was supported by the fellowship program of the International Agency for Research on Cancer and the European Union Co-funding of regional, national and international programs.

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